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     (FILE 'HOME' ENTERED AT 15:20:53 ON 04 OCT 2004)

     FILE 'BIOSIS, USPATFULL, EUROPATFULL, CAPLUS' ENTERED AT 15:21:24 ON 04
     OCT 2004
L1             4 S G!PROTEIN
L2         50809 S G-PROTEINS
L3          6222 S GPCR
L4          5590 S G-PCR
L5          7851 S FRET
L6           522 S BRET
L7             3 S L1 (L) L2
L8          1600 S L2 (L)L3
L9           236 S L3 (L) L4
L10          369 S L5 (L) L2
L11           27 S L6 (L) L4
L12           80 S L6 (L) L3
L13          149 S L8 (L) L5
L14           43 S L8 (L) L6
L15            1 S L13 AND PY <2000
L16            0 S L14 AND PY <2000
L17           22 S L13 AND PY<2003
L18            2 S L14 AND PY <2002
L19           16 S L10 AND PY<2001
L20           62 S L2 AND L6
L21           55 S L2 (L) L6
L22            2 S L21 AND PY <2001
L23           55 S L21 AND BRET
L24           39 S L23 AND HETEROTRIMERIC
L25            2 S L24 AND PY<2001
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L22 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Detection of beta2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET).
PY **2000**
AU Angers, Stephane; Salahpour, Ali; Joly, Eric; Hilairet, Sandrine; Chelsky, Dan; Dennis, Michael; Bouvier, Michel [Reprint author]
SO Proceedings of the National Academy of Sciences of the United States of America, (March 28, 2000) Vol. 97, No. 7, pp. 3684-3689. print.
CODEN: PNASA6. ISSN: 0027-8424.
AB Heptahelical receptors that interact with heterotrimeric **G proteins** represent the largest family of proteins involved in signal transduction across biological membranes. Although these receptors generally were believed to be monomeric entities, a growing body of evidence suggests that they may form functionally relevant dimers. However, a definitive demonstration of the existence of G protein-coupled receptor (GPCR) dimers at the surface of living cells is still lacking. Here, using bioluminescence resonance energy transfer (**BRET**), as a protein-protein interaction assay in whole cells, we unambiguously demonstrate that the human beta2-adrenergic receptor (beta2AR) forms constitutive homodimers when expressed in HEK-293 cells. Receptor stimulation with the hydrophilic agonist isoproterenol led to an increase in the transfer of energy between beta2AR molecules genetically fused to the **BRET** donor (Renilla luciferase) and acceptor (green fluorescent protein), respectively, indicating that the agonist interacts with receptor dimers at the cell surface. Inhibition of receptor internalization did not prevent agonist-promoted **BRET**, demonstrating that it did not result from clustering of receptors within endosomes. The notion that receptor dimers exist at the cell surface was confirmed further by the observation that BS3, a cell-impermeable cross-linking agent, increased **BRET** between beta2AR molecules. The selectivity of the constitutive interaction was documented by demonstrating that no **BRET** occurred between the beta2AR and two other unrelated GPCR. In contrast, the well characterized agonist-dependent interaction between the beta2AR and the regulatory protein beta-arrestin could be monitored by **BRET**. Taken together, the data demonstrate that GPCR exist as functional dimers in vivo and that **BRET**-based assays can be used to study both constitutive and hormone-promoted selective protein-protein interactions.

L13 ANSWER 1 OF 4 USPATFULL on STN DUPLICATE 1
AN 2003:67677 USPATFULL
TI Growth hormone secretagogue receptor family
IN Arena, Joseph P., Eagleville, PA, United States
Cully, Doris F., Scotch Plains, NJ, United States
Feighner, Scott D., Highlands, NJ, United States
Howard, Andrew D., Park Ridge, NJ, United States
Liberator, Paul A., Holmdel, NJ, United States
Schaeffer, James M., Westfield, NJ, United States
Van Der Ploeg, Leonardus H. T., Scotch Plains, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 6531314 B1 20030311
WO 9721730 19970619 <--
AI US 1998-77674 19980603 (9)
WO 1996-US19445 19961210
DT Utility
FS GRANTED
LN.CNT 1601
INCL INCLM: 435/325.000
INCLS: 536/023.100; 536/023.500; 530/350.000; 435/069.100; 435/320.100
NCL NCLM: 435/325.000
NCLS: 435/069.100; 435/320.100; 530/350.000; 536/023.100; 536/023.500
IC [7]
ICM: C12N015-00
EXF 530/350; 536/23.5; 536/23.1; 435/320.1; 435/325; 435/69.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 2 OF 4 USPATFULL on STN DUPLICATE 2
AN 2001:82525 USPATFULL
TI Assays for growth hormone secretagogue receptors
IN Pai, Lee-Yuh, Westfield, NJ, United States
Feighner, Scott D., Highlands, NJ, United States
Howard, Andrew D., Park Ridge, NJ, United States
Pong, Sheng-Shung, Edison, NJ, United States
Van Der Ploeg, Leonardus H. T., Scotch Plains, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 6242199 B1 20010605
WO 9722004 19970619 <--
AI US 1998-77675 19980603 (9)
WO 1996-US19442 19961210
19980603 PCT 371 date
19980603 PCT 102(e) date
PRAI US 1995-8582P 19951213 (60)
DT Utility
FS Granted
LN.CNT 1142
INCL INCLM: 435/007.200
INCLS: 435/007.210; 435/007.720; 435/069.100; 530/350.000; 536/023.100; 536/023.500
NCL NCLM: 435/007.200
NCLS: 435/007.210; 435/007.720; 435/069.100; 530/350.000; 536/023.100; 536/023.500
IC [7]
ICM: G01N033-566
EXF 435/7.2; 435/7.21; 435/66; 435/7.72; 435/69.1; 436/501; 530/350; 530/399; 530/300; 536/23.1; 536/23.5
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 3 OF 4 USPATFULL on STN DUPLICATE 3
AN 1999:102696 USPATFULL
TI Isolated nucleic acid molecules encoding a G-protein coupled receptor

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       showing homology to the 5HT family of receptors
IN     Glucksmann, M. Alexandra, Lexington, MA, United States
       Robison, Keith, Wilmington, MA, United States
PA     Millennium Pharmaceuticals, Inc., Cambridge, MA, United States (U.S.
       corporation)
PI     US 5945307             19990831                              <--
AI     US 1998-13634          19980126 (9)
DT     Utility
FS     Granted
LN.CNT 2826
INCL   INCLM: 435/069.100
       INCLS: 536/023.500; 435/252.300; 435/254.110; 435/320.100; 435/325.000
NCL    NCLM:  435/069.100
       NCLS:  435/252.300; 435/254.110; 435/320.100; 435/325.000; 536/023.500
IC     [6]
       ICM: C12N015-12
       ICS: C07K014-705
EXF    536/23.5; 435/69.1; 435/320.1; 435/325; 435/352.3; 435/254.11
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13  ANSWER 4 OF 4  USPATFULL on STN                    DUPLICATE 4
AN     1999:43394  USPATFULL
TI     Methods of assaying receptor activity and constructs useful in such
       methods
IN     Barak, Lawrence S., Durham, NC, United States
       Caron, Marc G., Hillsborough, NC, United States
       Ferguson, Stephen S., London, Canada
       Zhang, Jie, Durham, NC, United States
PA     Duke University, Durham, NC, United States (U.S. corporation)
PI     US 5891646             19990406                              <--
AI     US 1997-869568         19970605 (8)
DT     Utility
FS     Granted
LN.CNT 1569
INCL   INCLM: 435/007.200
       INCLS: 536/023.400; 530/350.000; 435/079.100; 435/069.100
NCL    NCLM:  435/007.200
       NCLS:  435/007.100; 435/069.100; 530/350.000; 536/023.400
IC     [6]
       ICM: G01N033-52
       ICS: C07H021-04; C12N015-12; C07K014-00
EXF    435/71; 435/174; 435/183; 435/6; 435/7.2; 435/69.1; 536/23.4; 536/23.5;
       530/350
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


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     (FILE 'HOME' ENTERED AT 14:12:18 ON 07 OCT 2004)

     FILE 'CAPLUS, MEDLINE, BIOSIS, USPATFULL' ENTERED AT 14:12:46 ON 07 OCT
     2004
L1       151748 S G PROTEIN?
L2         7783 S GPCR
L3        36430 S GFP
L4          583 S BRET
L5        20804 S BIOLUMINESCEN?
L6         2671 S L1 (L) L3
L7         2012 S L1 (L) L5
L8          512 S L2 (L) L3
L9          501 S L2 AND L5
L10         432 S L2 (L) L5
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L11             4 S L9 AND PY<2000
L12             4 S L10 AND PY<2000
L13             4 DUP REM L11 L12 (4 DUPLICATES REMOVED)

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=> d his

     (FILE 'HOME' ENTERED AT 17:08:25 ON 04 OCT 2004)

     FILE 'BIOSIS, CAPLUS' ENTERED AT 17:08:51 ON 04 OCT 2004
L1        42483 S G PROTEINS
L2        42483 S G-PROTEINS
L3        20914 S GFP
L4         3386 S FRET
L5           67 S L2 AND L4
L6           35 S L2 (L) L4
L7         4462 S GPCR
L8           49 S L7 AND L4
L9            7 S L8 (L)L6
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L9 ANSWER 1 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Development of a **FRET**-based system for studying G protein receptor-GIRK signaling.
AU Fowler, Catherine E. [Reprint Author]; Suen, Ka Fai [Reprint Author]; Slesinger, Paul [Reprint Author]
SO Biophysical Journal, (January 2004) Vol. 86, No. 1, pp. 444a-445a. print. Meeting Info.: 48th Annual Meeting of the Biophysical Society. Baltimore, MD, USA. February 14-18, 2004. Biophysical Society. ISSN: 0006-3495 (ISSN print).
AB Gbetagamma subunits liberated upon stimulation of G protein-coupled receptors (**GPCR**) bind to and activate GIRK channels. Although most combinations of Gbetagamma can activate GIRK channels, only **GPCRs** that couple via Gi/Go **G proteins** activate GIRK in native cells. We hypothesize this receptor specificity is established, in part, by the formation of membrane compartments in which GIRK channels coexist with the appropriate G protein and **GPCRs**. To address this, we will use **FRET** to study G protein-GIRK signaling in real-time. **FRET** is a highly sensitive technique well-suited to studying protein-protein interactions in living cells. To begin, we have made a series of YFP and CFP ('GFP') tagged constructs suitable for **FRET**, including the GABAB1, GABAB2 and mu opioid **GPCRs**, Galphao and Gbeta1 **G proteins** , and GIRK1 and GIRK2 channels. The function of these constructs was verified in transiently transfected HEK293T cells. Presence of the GFP tag did not appear to grossly alter channel or **GPCR** function, as determined by examining the agonist induced activation of GIRK currents using the whole-cell patch-clamp technique. The Galphao-GFP function was tested by introducing a mutation which rendered the Galphao-GFP insensitive to Pertussis toxin (Ptx). The Ptx-insensitive version of Galphao-GFP restored the coupling of GABAB receptors to GIRK channels in Ptx-treated cells; Ptx treatment (200ng/nl; 4h) abolished baclofen-induced currents in control cells transfected with Galphao-GFP (0.1 pA/pF+-0.3; n=4) but not in cells transfected with a Ptx-insensitive Galphao-GFP (102 pA/pF+-45; n=5). We conclude the Galphao-GFP is functional in HEK293T cells. Wide-field fluorescence microscopy indicated that GFP-tagged proteins can be visualized on the membrane surface. We are now testing the ability of the functional constructs to undergo **FRET** with each other under basal and stimulated conditions using evanescent wave microscopy.

L9 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Activated Gi proteins do not dissociate in intact cells.
AU Buenemann, Moritz [Reprint Author]; Frank, Monika [Reprint Author]; Lohse, Martin J. [Reprint Author]
SO Biophysical Journal, (January 2004) Vol. 86, No. 1, pp. 263a. print. Meeting Info.: 48th Annual Meeting of the Biophysical Society. Baltimore, MD, USA. February 14-18, 2004. Biophysical Society. ISSN: 0006-3495 (ISSN print).
AB Despite playing central roles in transducing extracellular signals into cellular responses no method was available to directly monitor G protein activity in intact cells. We developed a **FRET** assay using various CFP- and YFP-tagged mammalian G protein subunits and studied Gi protein activation in intact cells. Co-expression of Galphai-YFP (YFP was inserted into the alphahelical domain of Gai1) and either Ggamma2-CFP (N- or C-terminally tagged) or CFP-N-Gbeta1 resulted in detectable **FRET** between CFP and YFP either determined by donor dequenching or **FRET** ratio, recovery after acceptor photobleaching led to a fast **FRET** change which was complete within 1-2s. Stimulation of co-expressed alpha2A-adrenergic receptors led to a **FRET** **G proteins** play critical roles in determining specificity and kinetics of subsequent biological responses by modulation

of effector proteins. We have developed a **FRET** based assay to directly measure mammalian G protein activation in intact cells and found that Gi proteins activate within 1-2 s, which is considerably slower than activation kinetics of **GPCRs** themselves. More importantly, **FRET** measurements demonstrated that Galphai and Gbetagamma subunits do not dissociate during activation as has been previously postulated. Based on **FRET** measurements between Galphai-YFP and Gbetagamma subunits that were fused to CFP at various positions we conclude that instead G protein subunits undergo a molecular rearrangement during activation. The detection of a persistant heterotrimeric composition during G protein activation will impact the understanding of how **G proteins** achieve subtype selective coupling to effectors. This will be of a particular interest for unravelling Gbetagamma-induced signalling pathways.

L9 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI G-protein-coupled receptors function as oligomers in vivo.
AU Overton, Mark C.; Blumer, Kendall J. [Reprint author]
SO Current Biology, (March 23, 2000) Vol. 10, No. 6, pp. 341-344. print.
CODEN: CUBLE2. ISSN: 0960-9822.
AB Hormones, sensory stimuli, neurotransmitters and chemokines signal by activating G-protein-coupled receptors (**GPCRs**). Although **GPCRs** are thought to function as monomers, they can form SDS-resistant dimers, and coexpression of two non-functional or related **GPCRs** can result in rescue of activity or modification of function. Furthermore, dimerization of peptides corresponding to the third cytoplasmic loops of **GPCRs** increases their potency as activators of **G proteins** in vitro, and peptide inhibitors of dimerization diminish beta2-adrenergic receptor signaling. Nevertheless, it is not known whether **GPCRs** exist as monomers or oligomers in intact cells and membranes, whether agonist binding regulates monomer-oligomer equilibrium, or whether oligomerization governs **GPCR** function. Here, we report that the alpha-factor receptor, a **GPCR** that is the product of the STE2 gene in the yeast Saccharomyces cerevisiae, is oligomeric in intact cells and membranes. Coexpression of receptors tagged with the cyan or yellow fluorescent proteins (CFP or YFP) resulted in efficient fluorescence resonance energy transfer (**FRET**) due to stable association rather than collisional interaction. Monomer-oligomer equilibrium was unaffected by binding of agonist, antagonist, or G protein heterotrimers. Oligomerization was further demonstrated by rescuing endocytosis-defective receptors with coexpressed wild-type receptors. Dominant-interfering receptor mutants inhibited signaling by interacting with wild-type receptors rather than by sequestering G protein heterotrimers. We suggest that oligomerization is likely to govern **GPCR** signaling and regulation.

L9 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
TI Fluorescence resonance-detection of agonist-induced phospholipase C activation in live cells by analyzing the translocation of pleckstrin homol. domain-tagged protein containing fluorescent domains
IN Jalink, Kees
SO PCT Int. Appl., 94 pp.
CODEN: PIXXD2
AB The invention provides membrane mol. indicators, including polypeptides, encoding nucleic acid mols. and cells containing such polypeptides and nucleic acid mols. The invention membrane mol. indicators are characterized in that fluorescence resonance energy transfer (**FRET**) between a donor fluorescent domain and an acceptor fluorescent domain indicates a property of the membrane mol. The invention is exemplified using a pair of chimeric proteins called PLCδ1PH-CFP or PLCδ1PH-YFP containing pleckstrin homol. domain (as membrane mol. indicator domain-MMID, enabling

PIP2-binding) from phospholipase C δ1 and fluorescent domain from either cyan fluorescent protein or yellow fluorescent protein (CFP or YFP, as donor or acceptor). In resting cells, PH-CFP and PH-YFP reside at the plasma membrane bound to PI[4,5]P2 in the recombinant host cell, and the two fluoriphores remain within resonance distance. Upon activation of PLC by the addition of bradykinin (BK), PI[4,5]P2 is rapidly hydrolyzed and consequently PH domains of these proteins can no longer bind to the plasma membrane. Fluorescence resonance energy transfer between these plasma membrane-localized PLCδ1PH-CFP and PLCδ1PH-YFP in the recombinant host cell is used as a sensitive readout of phosphatidylinositol bisphosphate metabolism for monitoring agonist-induced phospholipase C activation. Anal. of the translocation responses suggests that localization of PLCδ1PH-CFP largely reports PI[4,5]P2 dynamics, although at high concns. IP3 can also contribute to translocation of the PH domains to the cytosol. Comparison of the Ca2+ and **FRET** -recorded responses of several agonists of **GPCRs** suggest that PLC activation detected by **FRET** is a more faithful reflection of receptor activity than the Ca2+ signal and that little if any desensitization or uncoupling occurs beyond the levels of **G proteins**. **FRET** detection of PLC activation is a fairly robust response and requires significantly less excitation intensity, enabling prolonged and fast data acquisition without the cell damage that limits confocal expts. It can be routinely obtained in a variety of cell types, especially motile or extremely flat cells. Other exemplary membrane mol. indicators containing PH domain and both fluorescence donor and acceptor domains in which **FRET** is low/high due to relocalization of membrane mol. and resulting separation/proximity of the donor and acceptor are also described. Also provided are methods of using the invention membrane mol. indicators to determine a property of a membrane mol., and to identify compds. that modulates a property of a membrane mol.

L9 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
TI Receptor mediated activation of heterotrimeric g-proteins
IN Devreotes, Peter N.; Janetopoulos, Chris
SO U.S. Pat. Appl. Publ., 13 pp.
CODEN: USXXCO
AB The invention concerns the receptor mediated activation of heterotrimeric **G-proteins** and their visualization in living cells by monitoring fluorescence resonance energy transfer (**FRET**) between subunits of a G protein fused to cyan and yellow fluorescent proteins. The G-protein hetero-trimer rapidly dissocs. and reassocn. upon addition and removal of cognate ligand. Energy transfer pairs of **G-proteins** enables direct in situ detection and have applications for drug screening and G protein coupled receptor (**GPCR**) de-orphaning.

L9 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
TI Receptor mediated activation of heterotrimeric G-proteins as monitored by fluorescence or bioluminescence resonant energy transfer
IN Devreotes, Peter N.; Janetopoulos, Chris
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
AB Receptor mediated activation of heterotrimeric **G-proteins** is visualized in living cells by monitoring fluorescence resonance energy transfer (**FRET**) between subunits of a G protein fused to cyan and yellow fluorescent proteins. The G-protein heterotrimer rapidly dissocs. and reassocs. upon addition and removal of cognate ligand. Energy transfer pairs of **G-proteins** enables direct in situ detection and have applications for drug screening and **GPCR** de-orphaning.

L9 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
TI G-protein-coupled receptors function as oligomers in vivo
AU Overton, Mark C.; Blumer, Kendall J.
SO Current Biology (2000), 10(6), 341-344
CODEN: CUBLE2; ISSN: 0960-9822
AB Hormones, sensory stimuli, neurotransmitters and chemokines signal by activating G-protein-coupled receptors (**GPCRs**) [1]. Although **GPCRs** are thought to function as monomers, they can form SDS-resistant dimers, and coexpression of two non-functional or related **GPCRs** can result in rescue of activity or modification of function [2-10]. Furthermore, dimerization of peptides corresponding to the third cytoplasmic loops of **GPCRs** increases their potency as activators of **G proteins** in vitro [11], and peptide inhibitors of dimerization diminish β2-adrenergic receptor signaling [3]. Nevertheless, it is not known whether **GPCRs** exist as monomers or oligomers in intact cells and membranes, whether agonist binding regulates monomer-oligomer equilibrium, or whether oligomerization governs **GPCR** function. Here, we report that the α-factor receptor, a **GPCR** that is the product of the STE2 gene in the yeast Saccharomyces cerevisiae is oligomeric in intact cells and membranes. Coexpression of receptors tagged with the cyan or yellow fluorescent proteins (CFP or YFP) resulted in efficient fluorescence resonance energy transfer (**FRET**) due to stable association rather than collisional interaction. Monomer-oligomer equilibrium was unaffected by binding of agonist, antagonist, or G protein heterotrimers. Oligomerization was further demonstrated by rescuing endocytosis-defective receptors with coexpressed wild-type receptors. Dominant-interfering receptor mutants inhibited signaling by interacting with wild-type receptors rather than by sequestering G protein heterotrimers. We suggest that oligomerization is likely to govern **GPCR** signaling and regulation.